Regulation of Cell Cycle

Introduction

The cell goes in a continuous and unidirectional cycle, G_1 to S, S to G_2, G_2 to M, and M to G_1. In the G_1 area the cell decides either to take a break from proliferation (entering G_0) or keep proliferating (staying in G_1 and starting anew). We as humans can understand what the phases are for and rationalize what "makes sense" to have happen before the next phase starts. But cells aren't humans. They don't have reasoning. They just do. Which means, there has to be an extraordinarily elegant system that ensures the cell does what it's supposed to do in each phase, progresses only when everything checks out, and does things in the right order. This elegant system is known as **checkpoints**. Checkpoints are hard stops. If the checkpoints are not satisfied, the cell cannot pass forward, and may even undergo apoptosis.

In the S phase, the DNA doubles. The cell needs to know that the DNA has doubled, there aren't any errors, and it's safe to progress to the G_2 phase. That's a checkpoint.

In the G_2 phase, the cell's cytoplasm needs to double, and organelles need to be replicated so that when mitosis happens, both daughter cells will live. G_2 is the last chance to give the DNA a once-over, to make sure there aren't any errors. The cell should only enter mitosis if all of these things happen. That's a checkpoint.

In the M phase A LOT happens. But most importantly, chromosomes line up on the metaphase plate. This way there's equal separation of genetic material to the two daughter cells. Before anaphase starts, the cell needs to ensure that the genetic material is ready to be split. That's a checkpoint.

But the checkpoint we are going to give the most attention to is the one between G_1 and S. G_1 can be any duration, but once the G_1 checkpoint is passed, the cell will either go on to mitosis or it will die. G_1 is the phase where the cell will produce receptors, transducer molecules, growth factors, transcription factors, etc. Proto-oncogenes will drive the cell to proliferation, and tumor suppressors will slow it down. While checkpoints do occur at various points, the most important and the most understood is the **G_1-S checkpoint**.

Phase	Activity	Cell Cycle Checkpoints
G_0	Gene expression of the cell	None, not inside the cell cycle
G_1	Prepare for synthesis—make proteins needed for synthesis phase	Is proliferation appropriate? (trophic signal) Is all the machinery ready for replication?
S	Replicate DNA	Did DNA get replicated entirely? Did DNA get replicated only once? Is DNA repair needed?
G_2	Prepare for division—double cytoplasm and organelles	Cytoplasm sufficient? Organelles sufficient? DNA check one last time
M	Division, between metaphase and anaphase	Is the metaphase plate aligned? Are the microtubules in place?

Table 6.1: Checkpoints and Cell Cycle Phases
Aligning the cell cycle phase with the teleologic goal of that phase, and therefore the teleologic purpose of the checkpoints.

A **proto-oncogene** is a gene that is pro-proliferation. **Gain of function** of a proto-oncogene results in cancer. Proto-oncogenes listen for the go signal; they are activated when things go right. When activated, they cause cell growth and proliferation.

A **tumor suppressor** is a gene that is anti-proliferation. **Loss of function** of tumor suppressors results in cancer. Tumor suppressors watch for damage, a problem, or a failure. They are activated when something goes wrong. They either arrest the cell cycle or induce apoptosis.

Cell Division Cycle Genes Control the Checkpoints

There are many regulators of cell cycle progression. Each checkpoint has a different final target. But at every checkpoint the gene products that give the advance-to-the-next-phase signal are the products of **cell division cycle** (CDC) **genes**. CDC genes include (but aren't limited to) **cyclins** and **cyclin-dependent kinases (CDKs)**.

CDKs are **constitutively expressed—**the gene is always making the same amount of CDK regardless of the cell cycle phase. And while the CDK levels remain constant, **CDK activity varies.** CDK activity variation is based on the amount of cyclin expression. **Cyclins are variably expressed**. It is by controlling cyclin expression that allows the cell to control its own cell cycle.

CDKs are **kinases**. Kinases phosphorylate things. At the end of each phase there is a checkpoint with a closed, locked door. If the CDKs are active, they **phosphorylate** the door and thereby **open the door**, allowing passage on to the next cell cycle phase. If the CDKs are not active, the door stays closed and the cell cycle cannot progress. More specifics on what "the door" is, below.

Cyclins activate CDKs. Cyclin expression is dependent on the tug and pull between tumor suppressor genes downregulating cyclin expression and proto-oncogenes upregulating cyclin expression. There is a **multi-gene cascade** on either side. This is intentional. If cyclins are expressed, CDK activity increases, and the cell moves forward in the cycle. Having multiple pro-proliferation proto-oncogenes involved ensures that a cell will only progress after all of the things that should have happened actually happen. Having multiple anti-proliferation tumor-suppressor genes ensures that multiple errors are searched for, and if there is any error at all, the cell cycle arrests. It's both the **combination of multiple genes** and the **sequence of gene expression** that ensures that the cell makes the right move.

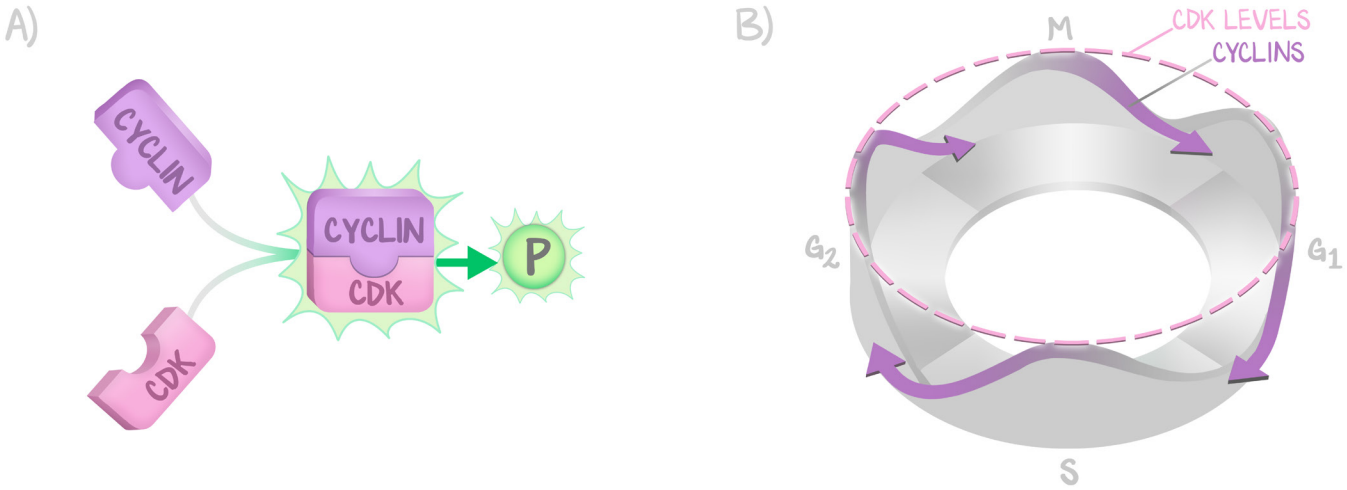

Figure 6.1: Cyclins and CDKs
(a) Cyclin-dependent kinases are constitutively expressed and activated by the presence of cyclins. (b) Cyclin expression is controlled by extrinsic factors, such as growth factors and other proto-oncogenes. The order of cyclin expression is fixed, such that the cyclin of G_1 both allows passage through the checkpoint but also initiates transcription of the next phase. Thus, the cell cycle is unidirectional.

Cyclin and CDK Example: Retinoblastoma

Retinoblastoma is the name of a cancer, but it's also the name of a cell cycle regulator gene that is dysfunctional in that cancer. This is NOT a discussion of the cancer retinoblastoma, but instead of the gene retinoblastoma (Rb).

The retinoblastoma gene (Rb) codes for a protein called retinoblastoma. The gene product of Rb, the retinoblastoma protein in its natural **dephosphorylated state, binds to** and **inhibits** a transcription factor E2F. E2F is THE final signal that progresses G_1 into S. If ever the retinoblastoma protein is **phosphorylated**, it will no longer bind to E2F, releasing E2F to bind its DNA element, advancing the cell cycle. This **one step** is what takes the cell from G_1 to S. If for any reason that retinoblastoma protein gets phosphorylated, it will release E2F, and the cell cycle advances.

What should happen is that as **cyclin D levels rise**, more cyclin D binds to more **CDK**, and that combination of **cyclin D + CDK** activates the kinase of the CDK, phosphorylating retinoblastoma, releasing E2F.

↑Cyclin D = ↑Cyclin-D-CDK = ↑Rb-P—E2F is NOT bound by Rb, disinhibiting cell cycle progression

↓Cyclin D = ↓Cyclin-D-CDK = ↑Rb—E2F is bound by Rb, inhibiting cell cycle progression

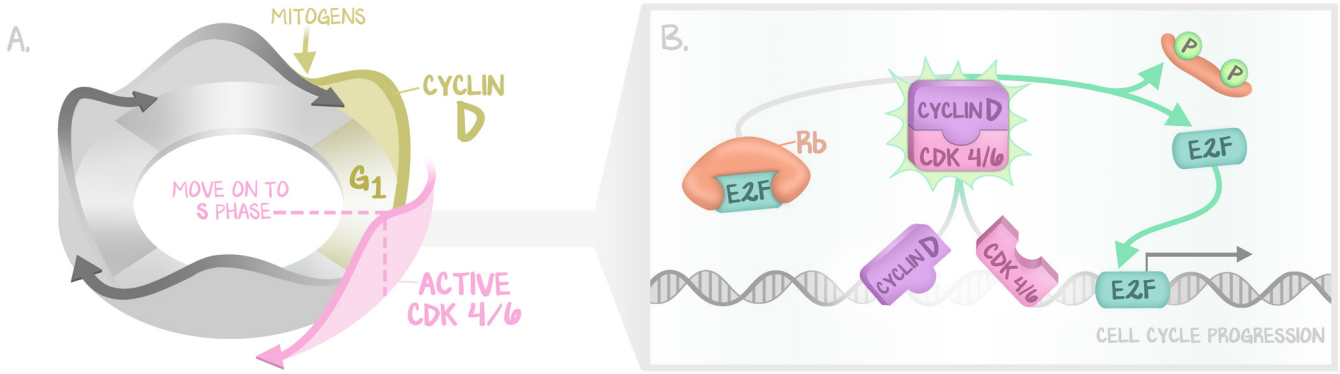

Figure 6.2: The G_1/S Checkpoint: Retinoblastoma
(a) If expressed in sufficient quantity, cyclin D will activate enough cyclin-dependent kinase to "open the door," by phosphorylating retinoblastoma. (b) In the nonphosphorylated state, the active Rb protein binds to and inactivates the transcription factor E2F. The phosphorylated state of Rb induces a conformational change, which leads to release of E2F from Rb. E2F is the transcription factor that initiates cell cycle progression and starts transcription of the genes for synthesis.

Proto-Oncogenes: The Sequence and the "Go" Signal

CDKs are always around, and in the same amount. Cyclin expression varies and so CDK activity varies. CDK-cyclin is the "go" signal that pushes the cell through a checkpoint in the cell cycle. But to get the cyclin expressed in the first place requires a sequence of events that ensures that a cell has the ability to regulate itself. Once cyclin is made, it activates CDK. When CDK acts, cyclins are cleared. Which means that in order to get the CDK-cyclin complex in sufficient quantity to activate cell cycle progression, the gene expression of cyclin must be greater than the degradation of cyclin. Cyclin genes are expressed in response to **growth factors.**

These genes are **growth factors** (PDGF), **growth factor receptors** (HER2/neu in breast cancer), **signal transducers** (ABL is the gene that codes for the JAK/STAT receptor in CML, caused by a translocation product BCR-ABL), **nuclear regulators** (transcription factors), and finally the **cell cycle regulators** (cyclin, CKD). Learning which is which is not worth the effort. Knowing which are active in a given cancer is. Just recognize that a cell must first express the receptor to a growth factor, receive a growth factor signal, transduce it to the cytoplasm, express a transcription factor and activate it—and only then can the cyclin gene be activated. But remember, it isn't just on or off. It's on in a sufficiently induced amount to overcome the elimination of the cyclin gene product.

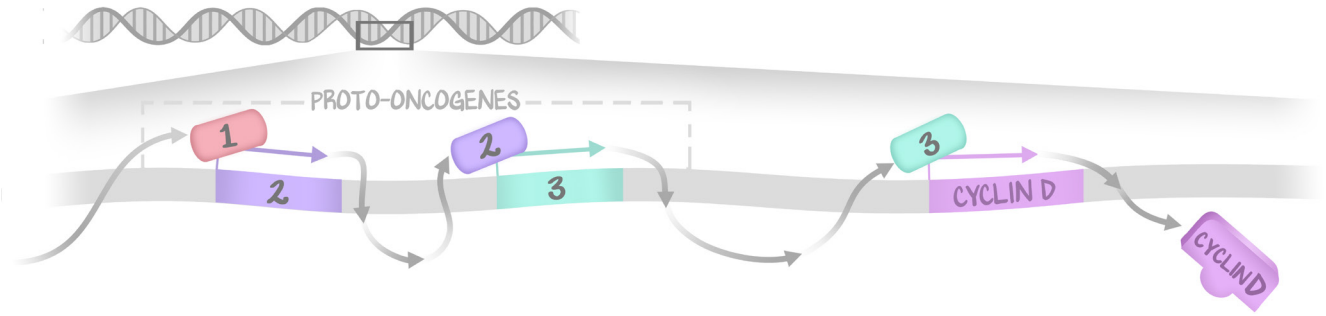

Figure 6.3: Progression of Oncogenes
The cell is able to ensure that everything is in order to progress to the next cell cycle with the progression of oncogenes. Those least related to the cell cycle progression are transcribed. This then leads to the receipt of a signal or an alteration of gene products that progresses.

Proto-oncogenes are every gene described in the sequential activation above. Proto-oncogenes are genes that when expressed **promote cell division**. They are called proto-oncogenes (as in pro-cancer) because if these mutate to **gain function**, then the cell will be pushed into proliferation because the sequence will progress forward from that oncogene even without the appropriate signal upstream in the sequence. A proto-oncogene becomes an **oncogene** when it gets mutated.

Tumor Suppressors: The "Stop" Signal

Tumor-suppressor genes are those that code for the breaks of the cell cycle. When expressed, they **arrest the cell cycle** or even **induce apoptosis.** Tumor-suppressor genes are the fail-safes, the backups. While there must be a sequence of positive signals (the proto-oncogenes), those positive signals don't look for problems. Tumor suppressors act independently of the proto-oncogene sequence. They look for problems, breaks in DNA, a failure of some step. They search for the negatives and give a negative signal if one is found. Mutations in tumor-suppressor genes must be a **loss of both alleles**, because they are **loss-of-function** mutations. Tumor-suppressor gene activation results in the expression of the stop signal, and either copy of that gene can do it. Therefore, both copies must be lost not to be able to express any stop signal.

The Rb gene we saw above is a tumor suppressor. **p53** is the other major tumor suppressor worth knowing. It's known as the **guardian of the genome**. We saw p53 in #3 *Apoptosis*; we include it here in review.

When DNA is damaged, ATM binds to the damaged DNA. ATM-binding-to-damaged-DNA activates the **ATM kinase**. ATM kinase adds a phosphate to Chk2 and to p53. Chk2-P also phosphorylates p53. Normally, **MDM inhibits p53**. But when p53 is phosphorylated, p53 is released. p53 acts as a transcription factor for other proteins. In **low concentrations** (the amount of DNA damage is low or time spent repairing has been short), p53 activates only **high-affinity receptors**, which causes **cell cycle arrest** and expression of **DNA repair genes**. In moderate concentrations, p53 induces

senescence—silencing all proto-oncogenes, preventing the cell from ever entering the cell cycle again. At **high concentrations**, p53 activates **low-affinity receptors**, which activate transcription of pro-apoptotic genes (Bax: pro-apoptosis; PUMA: anti-Bcl-2). To be clear, ATM, Chk2, Bax, and p53 are all proteins that either arrest the cell cycle or induce apoptosis and are therefore all tumor suppressors.

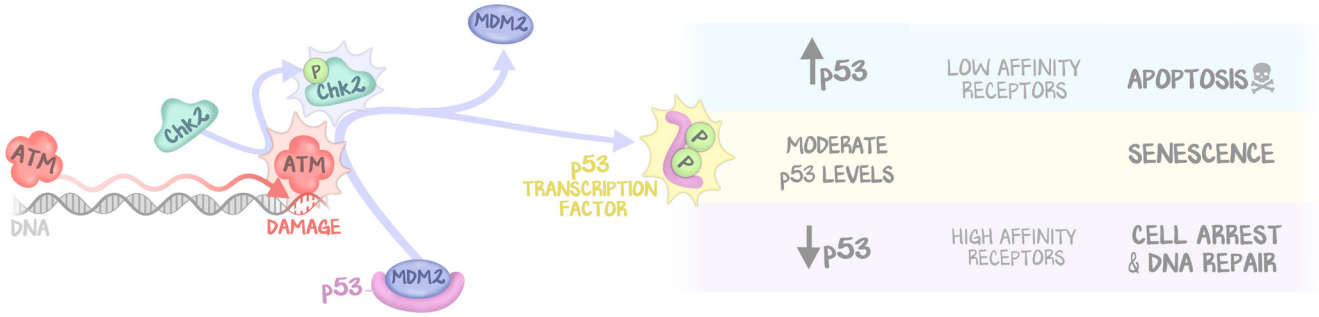

Figure 6.4: p53 Mechanism
At baseline, p53 is sequestered and not phosphorylated. Kinases activated by damaged DNA (ATM) or by ATM-kinase (Chk2) phosphorylate p53, releasing it from MDM2. The more p53 that is phosphorylated, the more likely it is to activate low-affinity receptors. p53 can result in cell arrest and DNA repair gene transcription, cell senescence, or apoptosis, depending on the level of activity.

The G_1 checkpoint is regulated by both the **Rb gene on chr 13** and the **p53 gene on chr 17**.

Radiation Sickness

This is why radiation sickness takes time to kill a person. The radiation causes catastrophic damage to all the cells in the body. But it doesn't kill the cell. When a cell tries to divide, its inner machinery determines there is too much DNA damage to repair, and so undergoes apoptosis. Rather than a hole bored through the body from a laser or a bullet, the radiation does its damage, then lets the cells' own machinery lead to organism death. There is no physical evidence of the exposure because the cells sacrifice themselves, realizing they should not propagate diseased DNA.